REMARKS

I. Status Summary

Claims 1-16, 26-28, and 42 were elected in response to a Restriction Requirement, and are now pending in the instant application.

In an Official Action dated August 10, 2005 (hereinafter the "Official Action"), the United States Patent and Trademark Office (hereinafter the "Patent Office") asserts that claims 14 and 15 have been withdrawn from consideration. There is no discussion as to why claim 14 has been withdrawn from consideration. Claim 15 has been withdrawn from consideration upon the assertion that it is not possible to judge whether peptides of SEQ ID NOs: 19 and 21 are related to the elected species, bovine gamma crystallin. Accordingly, the Patent Office has examined only claims 1-13, 16, 26-28, and 42.

The Sequence Listing has been objected to upon the contention that there are peptide and nucleic acid sequences in the specification, figures, and claims that do not have corresponding SEQ ID NOs.

The specification has been objected to upon the contention that it does not provide support for the SEQ ID NOs. recited in claims 12 and 15 or for the amino acid positions recited in claim 12.

Claims 1-13, 16, 26-28, and 42 have been rejected under 35 U.S.C. § 112, first paragraph, upon the contention that the phrase "located within" introduces new matter. Claims 1-13, 16, 26-28, and 42 have also been rejected under the written description and enablement requirements of 35 U.S.C. § 112, first paragraph.

Claims 1-6, 10, 11, 13, 16, 27, 28, and 42 have been rejected under 35 U.S.C. § 102(a) upon the contention that the claims are anticipated by Beste *et al.* (96 PNAS 1898-1903, 1999; hereinafter "Beste").

Claims 1, 2, 7-9, 11, 26, 27, and 42 have been rejected under 35 U.S.C. § 103(a) upon the contention that the claims are anticipated by Chirgadze *et al.* (D52 Acta Cryst 712-721, 1996; hereinafter "Chirgadze"). Claims 1, 2, 7-12, 26-28, and 42 have also been rejected under this section upon the contention that the claims are anticipated by den Dunnen *et al.* (Database PIR_79, Accession No. B24060; 189 *J Mol Biol* 37-46, 1986; hereinafter "B24060"); or den Dunnen *et al.* (Database PIR_79, Accession No.

A24060; 38 *Gene* 197-204, 1985; hereinafter "A24060"); or Graw *et al.* (Database UniProt, Accession No. P04344; 136 *Gene* 145-156, 1993; hereinafter "P04344");

The specification has been amended. Support for the amendments to the specification can be found throughout the specification itself, including particularly in the Figures (*e.g.*, Figures 1, 4, and 7-10) and in the Sequence Listing. Thus, no new matter has been added as a result of the amendments to the specification.

Claims 2 and 13 have been canceled without prejudice. Applicants respectfully reserve the right to file one or more continuation applications with claims directed to the subject matter of claims 2 and 13.

Claims 1 and 3-7 have been amended. Support for the amendments can be found throughout the specification of the application as filed, including particularly at page 4, lines 23-27 (new or improved binding properties). Additional support for the amendments can be found in the claims as filed (*e.g.*, original claim 2); in the Sequence Listing (bovine gamma-II-crystallin of SEQ ID NO: 22 and renumbering of amino acids in claim 12); and on page 8, lines 29-31 (amino acids located in at least two, three, or four beta strands).

New claim 46 has been added. Support for the new claim can be found throughout the specification as filed, including particularly in the claims as filed (*e.g.*, claims 1 and 21 as originally filed). Additional support can be found on page 6, lines 11-18 (exemplary proteins); page 8, lines 2-8 (selecting binding partners); page 8, line 15, through page 9, line 5 (mutagenesis); page 10, lines 4-5 (expression of the mutagenized protein); and original claim 24 (contacting the mutagenized protein and the binding partner and isolating mutagenized proteins).

Thus, no new matter has been added by virtue of the claim amendments or the addition of new claim 46. Reconsideration of the application as amended and based on the remarks set forth below is respectfully requested.

II. Response to the Withdrawal of Claims 14 and 15

The Patent Office has examined claims 1-13, 16, 26-28, and 42. In the Official Action, the Patent Office asserts that claim 15 was withdrawn from consideration upon the contention that it is not possible to judge whether peptides of SEQ ID NOs: 19 and

21 are related to the elected species, bovine gamma crystallin. The Patent Office further contends that claims 14, 17-25, 29-41, and 43-45 remain withdrawn from consideration.

Initially, applicants respectfully submit that claim 14 was never withdrawn from consideration by applicants. The Patent Office's attention is directed to the Response to the Restriction/Election Requirement filed November 19, 2004. In response to the Restriction/Election Requirement, applicants elected the claims of Group I, claims 1-16, 26-28, and 42, for prosecution. Thus, it appears that claim 14 is entitled to examination, and thus applicants respectfully request that the Patent Office reinstate claim 14.

With respect to claim 15, the Patent Office asserts that it is not possible to judge whether peptides of SEQ ID NOs: 19 and 21 are related to the elected species, bovine gamma crystallin, and thus has withdrawn this claim from consideration. Applicants respectfully disagree. Applicants respectfully submit that upon consideration of the specification and the Response to the Restriction/Election Requirement filed November 19, 2004, it is clear that applicants have elected bovine gamma crystallin as the species upon examination is to proceed.

Applicants further respectfully submit that the specification as filed clearly indicates that bovine gamma-II-crystallin was mutagenized to create a mutated protein that binds to estradiol and/or BSA-beta-estradiol 17-hemisuccinate. One such mutant, Mu 12A (*see* page 25, lines 1-15) is identified in the specification as having the amino acid sequence presented in Figure 8 (*see* page 25, line 11). The top sequence in Figure 8 is designated Mu 12A, and this sequence corresponds to SEQ ID NO: 19.

Similarly, page 25, lines 21-33, of the instant specification describe the amplification of the nucleic acid encoding Mu 12A and the subcloning of the amplification product into vector pET-20b. Page 25, lines 29-30, disclose that Figure 10 presents the amino acid sequence of Mu 12A and of gamma II-crystalline, respectively, after expression in pET 20b. Review of Figure 10 shows that the top sequence is identical to SEQ ID NO: 21. Applicants wish to point out that the Figure descriptions appearing on pages 14-16 of the instant specification have been amended to include reference to SEQ ID NOs. for all sequences disclosed in the Figures, as described in more detail hereinbelow.

As such, applicants respectfully submit that contrary to the Patent Office's contention, the specification indeed clearly discloses that SEQ ID NOs: 19 and 21 correspond to embodiments of the elected species. Therefore, applicants respectfully submit that the withdrawal of claim 15 from consideration should be reversed.

Accordingly, applicants respectfully submit that the withdrawal from consideration of claims 14 and 15 appears to be improper, and respectfully request that these claims be included in the examination of the instant application during the preparation of the next Official Action.

III. Response to the Objection to the Sequence Listing

An objection has been presented based on the Sequence Listing upon the contention that there are peptide sequences and nucleic acid sequences in the specification, figures, and claims that do not have corresponding SEQ ID NOs. Applicants respectfully submit that the specification has been amended to include SEQ ID NOs. where appropriate, including particularly in the Figure Descriptions beginning on page 14 and as shown hereinabove in the Amendments to the Specification.

More particularly, applicants respectfully submit that in accordance with Rule 821, several sections of the specification and the claims have been amended to recite specific SEQ ID NOs. Support for these amendments can be found in the Figures and the Sequence Listing, particularly Figures 1, 4, and 7-10, in view of the descriptions of these Figures presented in pages 14-16 and in the Examples. Additional support can be found on page 17, line 12 (bovine gamma-II-crystallin corresponds to GENBANK® Accession No. M16894, which encodes a protein that is identical to amino acids 1-175 of SEQ ID NO: 22). Thus, no new matter has been added by the amendments to the specification and Figures to include SEQ ID NOs.

Accordingly, applicants respectfully submit that the objection based on the Sequence Listing has been addressed, and respectfully request that it be withdrawn at this time.

IV. Response to the Objection to the Specification

The specification has been objected to upon the contention that the specification does not support the SEQ ID NOs. used in claims 12 and 15, or the amino acid positions recited in claim 12. After careful consideration of the objection, applicants respectfully traverse the objection and submit the following remarks.

Claim 12 recites the protein of claim 9, wherein at least one of the amino acids Lys 3, Thr 5, Tyr 7, Cys 16, Glu 18, Ser 20, Arg 37, and Asp 39 of a bovine gamma-II-crystallin of SEQ ID NO: 22 is mutagenized. Applicants respectfully submit that page 17, line 12, discloses that a nucleic acid sequence encoding bovine gamma-II-crystallin can be found at GENBANK® Accession No. M16894. The nucleotide sequence presented in GENBANK® Accession No. M16894 encodes a protein with the following amino acid sequence: MGKITFYEDRGFQGHCYECSSDCPNLQPYFSRCN SIRVDSGCWMLYERPNYQGHQYFLRRGDYPDYQQWMGFNDSIRSCRLIPQHTGTFR MRIYERDDFRGQMSEITDDCPSLQDRFHLTEVHSLNVLEGSWVLYEMPSYRGRQYLL RPGEYRRYLDWGAMNAKVGSLRRVMDFY. As can be seen, the amino acid at position 3 is Lys, the amino acid at position 5 is Thr, the amino acid at position 7 is Tyr, etc. Thus, the numbering presented in claim 12 for the positions of various amino acids in bovine gamma-II-crystallin is consistent with the known amino acid sequence for this polypeptide.

Applicants respectfully submit that the numbering presented in claim 12 is also consistent with the amino acid sequence of the recombinant bovine gamma-II-crystallin presented in SEQ ID NO: 22, which corresponds to the derived amino acid sequence of the bovine gamma-II-crystalline after subcloning into pET-20b. Page 25, lines 19-31, outline the construction of wild type bovine gamma-II-crystalline in pET-20b. As a result of the presence of restriction enzyme sites incorporated into the primers used to amplify the bovine gamma-II-crystalline prior to subcloning into pET-20b and the sequences present within the vector between the cloning sites employed and the His tag encoded by the vector, SEQ ID NO: 22 includes an additional 22 amino acids at the C-terminus, including the six His residues that comprise the His tag. The first 175 amino acids of SEQ ID NO: 22, however, are 100% identical to the complete amino acid sequence of bovine gamma-II-crystallin encoded by GENBANK® Accession No. M16894.

Thus, applicants respectfully submit that one of ordinary skill in the art upon consideration of the instant specification and the known amino acid sequence of bovine gamma-II-crystallin would understand that SEQ ID NO: 22 corresponds to wild type bovine gamma-II-crystallin to which a His tag has been added. Accordingly, applicants respectfully submit that claim 12, which recites the positions for mutagenesis of a protein that is in some embodiments a bovine gamma-II-crystallin through its dependency on claims 1 and 9, supports the recitation of SEQ ID NO: 22.

Furthermore, applicants respectfully submit that the discussion presented hereinabove with respect to the positions of the specific amino acids as recited in claim 12 and present in SEQ ID NO: 22 also addresses the objection to claim 12 with respect to the amino acid positions recited in claim 12. Therefore, applicants respectfully submit that the instant objections to the specification with respect to claim 12 have been addressed, and respectfully request that they be withdrawn at this time.

Turning now to the objection as applied to claim 15, applicants respectfully submit that claim 15 recites *inter alia* a protein with a new antigen binding specificity for a compound selected from the group consisting of estradiol and BSA-estradiol-17-hemisuccinate binding bovine gamma-II-crystalline mutants, two examples of which applicants respectfully submit have amino acid sequences comprising SEQ ID NOs: 19 or 21. Applicants respectfully submit that the specification fully supports the recitation of these SEQ ID NOs. in claim 15.

For example, the description of Figure 8 that can be found on page 15 of the instant specification indicates that Figure 8 presents the derived amino acid sequences of the BSA-estradiol-17-hemisuccinate-binding bovine gamma-II-crystalline mutant 12A (Mu 12A) and of gamma-II-crystalline wild type (WT) after expression in the phagemids and removal of the signal peptide. This recitation has been amended to recite that the sequence presented as **Mu 12A** corresponds to SEQ ID NO: 19. Similarly, the description of Figure 10 that can be found on page 16 of the instant specification indicates that Figure 10 presents the derived protein sequence of the BSA-estradiol-17-binding mutant 12A and of gamma-II-crystalline after expression in pET-20b. Here again, this recitation has been amended to recite that the sequence presented as **Mu 12A-His** corresponds to SEQ ID NO: 21. Support for these amendments can be found

in the Sequence Listing, which discloses the sequences as SEQ ID NOs: 19 and 21, respectively. Additional support can be found on page 25, lines 1-15, which describes the characterization of **Mu 12A**, and page 25, lines 19-31, which describes the characterization of **Mu 12A-His**.

Accordingly, applicants respectfully submit that the specification fully supports the recitations of SEQ ID NOs: 19 and 21 in claim 15. Applicants therefore respectfully request that the instant objection to the specification be withdrawn at this time. Applicants further respectfully submit that claims 12 and 15 are in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

V. Responses to the Rejections under 35 U.S.C. § 112, First Paragraph

Claims 1-13, 16, 26-28, and 42 have been rejected under 35 U.S.C. § 112, first paragraph, on three different bases. First, the Patent Office asserts that the phrase "located within" introduces new matter. Second, the Patent Office asserts that bovine gamma-II-crystallin is not a sufficient representative of the genus of beta-sheet comprising proteins recited in the claims, and further that the recited functional limitation does not provide sufficient structural characteristics to define the genus. And third, the Patent Office asserts that while the specification enables crystallin protein mutants obtained by phage display, it does not enable other proteins with beta-sheet structure in which amino acids located on the surface of beta strands are mutagenized.

After careful consideration of the rejections, applicants respectfully traverse the rejections and submit the following remarks.

V.A. Response to the New Matter Rejection

According to the Patent Office, the amendment of claims 1 and 3-6 to recite amino acids located "within" beta strands brings a distance limitation that was not described in the specification into the claims. Applicants respectfully submit, however, that the term "located within" is intended to indicate that the amino acids to be mutagenized are found in beta strands.

Nonetheless and without acquiescing to the Patent Office's assertions concerning the phrase "located within", in an effort to facilitate the prosecution of the pending claims, applicants have amended the phrase "located within" appearing in

claims 1 and 3-6 to "located in". Support for the amendments can be found throughout the specification as filed, including particularly at page 8, lines 29-31, which states: "The insertions, deletions or substitutions of one or more amino acids are located in at least two beta strands exposed on the surface of at least one beta-sheet exposed on the surface" (emphasis added).

Accordingly, applicants respectfully submit that the new matter rejection of claims 1-13, 16, 26-28, and 42 under 35 U.S.C. § 112, first paragraph, has been addressed, and respectfully request that it be withdrawn at this time. Claims 2 and 13 have been canceled, and thus the rejection is believed to be moot as to these claims. As such, applicants respectfully submit that claims 1, 3-12, 16, 26-28, and 42 are in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

V.B. Response to the Written Description Rejection

Claims 1-13, 16, 26-28, and 42 have also been rejected under the written description requirement of 35 U.S.C. § 112, first paragraph. The basis of this rejection appears to be that the specification discloses a mutated bovine gamma-II-crystallin, and that this disclosure is not sufficient to support the genus of all beta-sheet proteins.

Applicants respectfully disagree. Applicants have disclosed not only bovine gamma-II-crystallin, but have also disclosed crystallins generally, including alpha, beta, and gamma-crystallins, spherulins, heat shock proteins, cold shock proteins, beta-helix proteins, lipocalins, certins or transcription factors, fibronectins, GFP, NGF, tendamistat, and lysozyme as representative β-sheet-containing proteins that can be mutagenized using the disclosed methods (*see* page 6, lines 11-18).

Furthermore, applicants respectfully submit that the Patent Office's assertion that the "generally stated functional limitations do not provide sufficient structural characteristics to define the genus of claimed proteins" is likewise believed to be inaccurate given the breadth of the disclosure when considered from the perspective of one of ordinary skill in the art. Applicants respectfully submit that all of the proteins encompassed by claim 1 are related to each other with regard to their structures, which can be modified in accordance with the disclosed methods.

For example, alpha-, beta-, and gamma-crystallins are highly homologous regarding their structure. They are all proteins of the eye lens, and are highly similar at

the amino acid sequence, functional, and structural levels. Gamma-crystallins, for example, include two domains with a greek key motif as described on page 4, lines 1-11, of the instant specification. Beta-crystallins contain four greek key motifs, and are thus properly considered as "doubled" gamma-crystallins. Spherulins are small proteins with a single greek key motif that aggregates to form a dimer. Furthermore, according to the Structural Classification of Proteins (SCOP) database, spherulins structurally belong to the group of crystallins. Heat shock proteins often have an alpha-crystallin like domain with all beta-structure, cold shock proteins are small proteins (10 kDa) with all beta-structure, fibronectin is a small structural protein that like gamma-crystallin comprises a beta-sandwich and a greek key motif, and beta helix proteins are also all beta-proteins.

Summarily, the subject matter encompassed by present claim 1 is characterized by common structural features, and the recited proteins and are highly related to each other. As the presently disclosed subject matter is directed to modifications in these common structural elements (*e.g.*, at least two beta-strands), applicants respectfully submit that one of ordinary skill in the art would conclude upon consideration of the instant specification that the presently disclosed subject matter as exemplified by the modification of bovine gamma-crystallin can be generalized to other proteins with these common structural features. Considering the common structural elements of the proteins covered by claim 1 and contrary to the Patent Office's contentions, applicants respectfully submit that claim 1 encompasses a reasonable number of structurally related proteins that can be modified in these common structural elements to produce the claimed proteins.

Additionally, applicants respectfully submit that the following technical elements are defined in claim 1, and modifications in accordance with these technical elements result in a protein with new antibody-like antigenic binding activities as exemplified by bovine-gamma-II-crystallin. The following precisely defined features are common elements to all modified proteins: (a) the starting point is a protein with a beta-sheet structure; (b) the proteins are selected from crystallins, spherulins, heat shock proteins, cold shock proteins, beta helix proteins, and fibronectin; and (c) the proteins are mutagenized by an insertion, a deletion, a substitution, or a combination thereof.

Applicants further respectfully submit that the mutations are generated in clearly defined positions of the proteins. For example, the mutations are in a beta-sheet exposed to the surface of the protein, the mutations are introduced into at least two beta-strands that are exposed on the surface of the protein, and these two beta-strands are located within a beta-sheet of the protein.

As such, applicants respectfully submit that the presently disclosed subject matter is based at least in part on the new and surprising finding that a well-known class of proteins with beta-sheet structure can be modified in well defined regions of the protein (modifications of amino acids on a surface of the protein located in at least two beta-strands of at least one beta-sheet), and that these modifications can be used to create new binding properties in the proteins towards a defined target molecule so that the resulting protein is able to specifically bind to the target molecule. The new protein thus behaves like an antibody.

Applicants further respectfully submit that it was quite surprising that beta-sheets, which typically occur in structural proteins like crystallins and that applicants respectfully submit one of ordinary skill in the art would have believed to be rigid and poor candidates for modification to produce new binding activities, can be modified in order to produce such new binding activities. Thus, given the disclosure in the instant specification of a representative modification of a representative beta-sheet protein, one of ordinary skill in the art would understand that other beta-sheet-containing proteins could be similarly modified to produce mutagenized proteins with new binding activities.

Thus, applicants respectfully submit that the Patent Office's apparent requirement that embodiments of the presently disclosed subject matter other than a mutagenized bovine gamma-II-crystallin that binds BSA-estradiol-17-hemisuccinate be disclosed is believed to be improper. Rather, the written description requirement of 35 U.S.C. § 112, first paragraph, requires no more than that the specification demonstrate that applicants were in possession of the claimed subject matter, and applicants respectfully submit that this requirement has clearly been met.

Accordingly, applicants respectfully request that the rejection of claims 1-13, 16, 26-28, and 42 under the written description requirement of 35 U.S.C. § 112, first paragraph, be withdrawn. Claims 2 and 13 have been canceled, and thus the rejection

is believed to be moot as to these claims. As such, applicants respectfully submit that claims 1, 3-12, 16, 26-28, and 42 are in condition for allowance, and respectfully solicit a Notice of Allowance at this time.

V.C. Response to the Enablement Rejection

Claims 1-13, 16, 26-28, and 42 have also been rejected under the enablement requirement of 35 U.S.C. § 112, first paragraph. In support of this rejection, the Patent Office asserts that while the specification enables crystallin protein mutants obtained by phage display, it does not enable other proteins with beta-sheet structure in which amino acids located on the surface of beta strands are mutagenized.

Applicants respectfully submit that the instant rejection of claims 1-13, 16, 26-28, and 42 is improper. Initially, applicants respectfully submit that in order to enable product claims, applicants need only disclose a single method that would enable one of ordinary skill in the art to produce the claimed products, and that a lack of disclosure concerning other methods that can be employed does not render the claims non-enabled under 35 U.S.C. § 112. *See* M.P.E.P. § 2164.01(b); *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970); *Spectra-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 1533, 3 USPQ2d 1737, 1743 (Fed. Cir.), cert. denied, 484 U.S. 954 (1987).

Furthermore, the Patent Office concedes that the specification is enabling for crystallin protein mutants obtained by phage display. Applicants respectfully submit, however, that the specification enables more than just crystallin proteins mutants obtained by this technique. Applicants respectfully submit that the specification informs one of ordinary skill in the art of a considerable number of proteins that can be similarly mutagenized. Applicants further respectfully submit that the Patent Office has identified no basis for concluding that these other proteins would not also be expected to behave similarly to crystallin given the structural features identified in the specification that are shared among these proteins. According to *In re Marzocchi*, 439 F.2d 220, 169 USPQ 367 (CCPA 1971),

> As a matter of Patent Office practice, then, a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of

> the statements contained therein which must be relied on for enabling support.

In re Marzocchi at page 223 (emphasis added).

Furthermore, according to the Training Materials for Examining Patent Applications with Respect to 35 U.S.C. Section 112, First Paragraph-Enablement Chemical/Biotechnical Applications "the case law makes clear that properly reasoned and supported statements explaining any failure to comply with Section 112 are a requirement to support a rejection". *Citing In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). Applicants respectfully submit that no such "properly reasoned and supported statements" have been presented, other than general assertions, rebutted herein, that the claims encompass "any protein with beta-sheet structure" or other naturally occurring crystallins.

As a result, applicants respectfully submit that the Patent Office has not satisfied its burden concerning the establishment of a *prima facie* case of lack of enablement of claims 1-13, 16, 26-28, and 42.

Continuing with the instant rejection, applicants respectfully traverse the Patent Office's assertion that the subject matter encompasses "random protein[s] having beta sheet structure in which amino acids located on the surface beta strands". Rather, applicants respectfully submit that the specification includes extensive guidance as to the relevant characteristics of the starting proteins. For example, the specification discloses that:

> Proteins with beta-sheet structure are known. An example of a protein class with beta-sheet is the crystallins, in particular alpha- beta- and gamma-crystallins. It is in principle possible to use crystallins from all kinds of animals, for example from vertebrates, rodents, birds and fish. Further examples of proteins which have beta-sheet structure and can be mutagenized according to the invention are: spherulins, heat shock proteins, cold shock proteins, beta-helix proteins, lipocalins, certins or transcription factors, fibronectins, GFP, NGF, tendamistat or lysozyme. For example, individual subunits or domains of the said proteins, for example crystallins, which have beta-sheet structure, are mutagenized according to the invention.

Specification at page 6. The specification further discloses on page 8 that:

> Amino acids exposed on the surface are accessible to the surrounding solvent. If the accessibility of amino acids in a protein is more than 8% compared with the accessibility of the amino acid in the model tripeptide Gly-X-Gly, these amino acids are called amino acids exposed on the surface. These protein regions or individual amino acid positions are also preferred binding sites for possible binding partners which are to be selected for according to the invention. The binding partners may be, for example, antigens or substrates or substrate-transition-state analogues.
>
> According to the invention, it is possible to mutagenize virtually all proteins which display beta-sheet structures located on the surface and accessible to a solvent or a binding partner. To this end, suitable proteins are mainly those which are particularly stable, i.e. resistant to denaturation, for example, or sufficiently "small".

Specification at page 8.

Thus, applicants respectfully submit that the specification as filed provides a context from which one of ordinary skill in the art could select individual proteins for mutagenesis, and further that these proteins are ones that "display beta-sheet structures located on the surface and accessible to a solvent or a binding partner" including, but not limited to spherulins, heat shock proteins, cold shock proteins, β-helix proteins, lipocalins, certins or transcription factors, fibronectins, GFP, NGF, tendamistat, and lysozyme (*see* Specification at page 6). As such, applicants respectfully submit that the specification provides considerable direction and guidance as to the class of proteins that can be mutagenized to produce the presently disclosed subject matter.

Applicants also respectfully traverse the Patent Office's apparent assertion that the specification only enables the isolation of mutants by phage display. For example, page 9, line 13, to page 10, line 16, of the instant specification discloses different methods for mutagenizing proteins as well as representative expression systems. Additionally, page 11, Heading Numbers 2 and 3 describe methods other than phage display that can be employed for detecting mutated proteins. Heading Number 4 describes expression and secretion methods for the mutated proteins, particularly on page 14 of the instant specification.

Accordingly, applicants respectfully submit that the specification discloses alternative methods to phage display, and upon consideration of the instant

specification, one of ordinary skill in the art would be able to perform these and other methods to create, express, and isolate mutant proteins without undue experimentation.

Summarily, applicants respectfully submit that the instant specification complies with the enablement requirement of 35 U.S.C. § 112, first paragraph, because there is considerable direction and guidance provided to one of ordinary skill in the art in the subject U.S. patent application as filed. *See In re Wands*, 858 F.2d 736, 740, 8 USPQ2d 1403, 1406 (Fed. Cir. 1988). Thus, applicants respectfully request that the instant rejection of claims 1-13, 16, 26-28, and 42 under the enablement requirement of 35 U.S.C. § 112, first paragraph, be withdrawn. Claims 2 and 13 have been canceled, and thus the rejection is believed to be moot as to these claims. Therefore, applicants respectfully submit that claims 1, 3-12, 16, 26-28, and 42 are in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

VI. Responses to the Rejections under 35 U.S.C. § 102

Claims 1-13, 16, 26-28, and 42 have been rejected under 35 U.S.C. § 102(a) and/or 102(b) upon the contention that the claims are anticipated by one or more of Beste, Chirgadze, B24060, A24060, and P04344. After careful consideration of the rejections and the Patent Office's bases therefor, applicants respectfully traverse the rejections and submit the following remarks.

VI.A. Response to the Rejection over Beste

Claims 1-6, 10, 11, 13, 16, 27, 28, and 42 have been rejected under 35 U.S.C. § 102(a) upon the contention that Beste anticipates the claims. According to the Patent Office, Beste teaches a mutagenized lipocalin that has acquired a new feature – the ability to bind fluorescein. The Patent Office further asserts that the β-sheet proteins produced with the various mutations disclosed in Beste anticipate claims 3-6, 10, 11, 13, 16, 27, 28, and 42, and also that claim 16 is anticipated by the solution of the ELISA binding assay disclosed in Beste.

Applicants respectfully disagree. Applicants respectfully submit that claim 1 has been amended to recite *inter alia* that the protein to be mutagenized is selected from the group consisting of a crystallin, a spherulin, a heat shock protein, a cold shock protein, a β-helix protein, and a fibronectin. Lipocalin is neither a crystallin, a spherulin, a heat

shock protein, a cold shock protein, a β-helix protein, or a fibronectin, and for this reason, applicants respectfully submit that Beste does not anticipate claim 1.

Furthermore, Beste at best teaches an engineered protein derived from a beta-sheet structured lipocalin, wherein the already existing antigen-binding specificity was altered in order to create a different ligand specificity. Beste does not describe the creation of a synthetic binding site *de novo* on the surface of a protein. Indeed, the lipocalin of Beste is a bilin-binding protein (BBP) and has, therefore, an already existing ligand-binding pocket. It is this already existing ligand-binding pocket that is mutagenized in Beste, and thus the mutagenized protein of Beste falls outside of proviso (i) recited in claim 1.

Additionally, applicants respectfully submit that Beste's mutagenized lipocalin does not satisfy proviso (ii) of claim 1. Proviso (ii) states "the protein has a binding activity before the substitution, deletion, or insertion, and that after the substitution, deletion, or insertion at the surface of the beta-sheet structure, the protein has an additional new or improved binding activity" (emphasis added). Applicants respectfully submit that the Patent Office's assertion that the lipocalin disclosed in Beste has acquired a new binding affinity does not equate to the additional new or improved binding activity recited in the claim.

To elaborate, applicants respectfully submit that the alteration of the binding affinity of lipocalin from biliverdin IX to fluorescein might be a new binding activity, but it does not provide the mutagenized lipocalin with an additional new property. Applicants respectfully submit that the recitation of the term "additional" in claim 1 indicates that the mutagenized protein does not lose the binding activity that it already had: in the case of Beste's lipocalin, the ability to bind biliverdin IX. Applicants respectfully submit, however, that the mutations presented in Beste would destroy the ability of the lipocalin to bind to biliverdin IX because the mutations are in the ligand-binding pocket itself.

Stated another way, claim 1 clearly recites in proviso (ii) that if the non-mutagenized protein has a binding activity, the mutagenesis creates an additional binding activity. The term "additional" in proviso (ii) indicates that the prior existing binding activity is maintained while a further new additional property is created *de novo* at a site different from the pre-existing binding pocket. This aspect of the presently

claimed subject matter is disclosed in the specification as filed on page 12, line 27, through page 13, line 2, which recites in part with respect to the definition of "protein having a new specific property" that "this also includes proteins which already had a specific binding property or a catalytic activity prior to mutagenization and, after mutagenization in the beta-sheet, possess another, additional specific binding property and/or catalytic activity" (emphasis added).

Summarily, applicants respectfully submit that the alteration of the biliverdin IX binding pocket that results in the ability of the mutagenized protein to bind fluorescein in this same binding pocket would render the lipocalin incapable of binding biliverdin IX. As a result, while the Beste lipocalin might have a new binding activity, it does not have an additional new binding activity.

Accordingly and for the reasons set forth hereinabove, applicants respectfully submit that Beste does not anticipate claim 1. Claims 2-6, 10, 11, 13, 16, 27, and 28 all depend directly or indirectly from claim 1, and thus are also believed to be distinguished from claim 1. Claims 2 and 13 have been canceled, and thus the rejection is believed to be moot as to these claims. With respect to claim 42, this claim recites a mutagenized gamma crystallin polypeptide, and since lipocalin is not a gamma crystallin, it is clear that Beste does not anticipate claim 42.

Thus, applicants respectfully submit that the instant rejection of claims 1, 3-6, 10, 11, 16, 27, 28, and 42 has been addressed, and further respectfully submit that these claims are now in condition for allowance. Applicants respectfully solicit a Notice of Allowance to that effect.

VI.B. Response to the Rejection over Chirgadze

Claims 1, 2, 7-9, 11, 26, 27, and 42 have been rejected under 35 U.S.C. § 102(b) upon the contention that the claims are anticipated by Chirgadze. According to the Patent Office, Chirgadze discloses a bovine gamma crystallin containing mutations in surface residues Leu 51, Ile 103, and His 155. The Patent Office thus asserts that the reference reads on a protein with β sheet structure in which surface amino acid residues located on at least 2 β-strands are mutated.

Initially, applicants respectfully submit that Chirgadze discloses the wild type sequences of bovine gamma B, gamma C, and gamma D crystallins. Thus, applicants

respectfully submit that none of the sequences disclosed in Chirgadze can properly be considered a protein with beta-sheet structure wherein amino acids on a surface of the protein located in at least two β-strands of at least one beta sheet are mutagenized as recited in claim 1. Irrespective of the reference in Chirgadze to "key mutations involving surface residues Leu51, Ile103, and His155" (*see* Chirgadze at page 712), applicants respectfully submit that it is clear that the present claims do not refer to such naturally occurring "mutations". Rather, applicants respectfully submit that the presently disclosed and claimed subject matter relates to proteins with beta-sheet structure that are engineered to have new binding specificities.

To elaborate, applicants respectfully submit that claim 1 recites *inter alia* that amino acids on a surface of the protein located in at least two β-strands of at least one beta sheet are mutagenized. Chirgadze discloses amino acid differences at Leu 51, Ile 103, and His 155. However, contrary to the Patent Office's assertion, these residues are not present on at least 2 beta-strands. **Exhibit A** presents a diagram of structure of bovine gamma B crystallin (pdb code 1AMM). Residues Leu 51, Ile 103, and His 155 are displayed as red sticks. These residues, which differ between gamma B and gamma D crystallin as described by Chirgadze, are either located in surface loops (*i.e.*, not in beta-strands) (Leu 51 and His 155; upper residues) or in the solvent-shielded part of one beta-strand (Ile 103, on the left).

The Patent Office's attention is also directed to **Exhibit B**, which presents a true and accurate copy of a graphical depiction of GENBANK® Accession No. 1ELPB, which corresponds to Chain B, Gamma-D Crystallin Structure At 1.95 Å Resolution. With particular reference to the depiction of secondary structure associated with the sequence at the bottom of **Exhibit B**, applicants respectfully submit that Leu 51 and His 155 are indicated as not being in beta-strand structure. Thus, applicants respectfully submit that the asserted disclosure in Chirgadze of amino acid differences between the bovine gamma B crystallin of SEQ ID NO: 22 and Leu 51, Ile 103, and His 155 of the bovine gamma D crystallin does not support the instant rejection because these residues do not represent amino acids on a surface of the protein located in at least two β-strands.

Furthermore, wild type crystallins are not believed to have any binding activities. Claim 1 also recites that the mutagenizing is selected from the group consisting of an insertion, a deletion, a substitution, and combinations thereof, such that the protein has a new or improved antigen binding specificity. Since wild type bovine gamma B, gamma C, and gamma D crystallins are not believed to have any antigen binding specificities, applicants respectfully submit that the disclosure of these proteins in Chirgadze does not support the instant rejection of claim 1.

Therefore, applicants respectfully submit that claim 1 has been distinguished over Chirgadze. Applicants further respectfully submit that claims 2, 7-9, 11, 26, 27, and 42 are also believed to be distinguished from Chirgadze based upon their direct or indirect dependency from claim 1. Claim 2 has been canceled, and thus the instant rejection as applied to claim 2 is believed to have been rendered moot. As a result, applicants respectfully request that the instant rejection of claims 1, 7-9, 11, 26, 27, and 42 over Chirgadze be withdrawn, and further that claims be allowed at this time.

VI.C. Response to the Rejections over B24060, A24060, *or* P04344

Claims 1, 2, 7-12, 26-28, and 42 have been rejected under 35 U.S.C. § 102(b) upon the contention that the claims are anticipated by B24060, A24060, or P04344. According to the Patent Office, B24060, A24060, and P04344 disclose gamma crystallins from rat, human, and mouse, respectively, which similar to the disclosure of Chirgadze summarized hereinabove, meet the structural limitations of the claims. The Patent Office further asserts that since each of the cited gamma crystallins is from a different source, it is assumed to have different binding capabilities to rat, human, and mouse antibodies, respectively.

Applicants respectfully traverse both of the Patent Office's assertions in support of the instant rejection. First, applicants respectfully traverse the Patent Office's assertion that the wild type amino acid sequences presented in GENBANK® Accession Nos. B24060, A24060, and P04344 meet the structural limitations of claim 1. Applicants respectfully submit that the remarks presented hereinabove with respect to Chirgadze are equally applicable to the instant rejection. Particularly, applicants respectfully submit that B24060, A24060, and P04344 disclose wild type crystallins, and

thus do not disclose the amino acid sequences of proteins that are mutagenized or that have any antigen binding activities as recited in claim 1.

Furthermore, applicants respectfully submit that the Patent Office's assumption that B24060, A24060, and P04344 have different binding capabilities to rat, human, and mouse antibodies, respectively, is scientifically inaccurate and disregards the "antigen binding specificity" element of claim 1.

To elaborate, applicants respectfully submit that B24060 discloses the amino acid sequence of rat gamma B crystallin, A24060 discloses the amino acid sequence of rat gamma A crystallin, and P04344 discloses the amino acid sequence of mouse gamma B crystallin. Thus, it appears that the reference to GENBANK® Accession No. A24060 is an error. It is possible that the Patent Office intended to refer to GENBANK® Accession No. P07316 (hereinafter "P07316"), which is human gamma B crystallin.

Continuing under the assumption that this was the intention of the Patent Office, applicants respectfully submit that rat gamma B crystallin, human gamma B crystallin, and mouse gamma B crystallin would have no binding activity towards rat, human, or mouse antibodies because each would be recognized by the host animal as self. Self-antigens are not reactive with antibodies from that species, and as such, applicants respectfully submit that the Patent Office's assumption that the polypeptide disclosed in GENBANK® Accession No. B24060 would bind to rat antibodies, the polypeptide disclosed in GENBANK® Accession No. P07316 would bind to human antibodies, and the polypeptide disclosed in GENBANK® Accession No. P04344 would bind to mouse antibodies is unsupportable.

Furthermore, even if each crystallin were to be bound by different antibodies, applicants respectfully submit that being bound by an antibody (*i.e.*, having an antigen-like quality) is not equivalent to having an antigen-binding activity as recited in claim 1. Stated another way, the presently disclosed subject matter relates to mutagenized proteins that act like antibodies: meaning that they bind to other molecules. This is not equivalent to be bound by other molecules such as antibodies. Applicants respectfully submit that in order to support the instant rejection, the Patent Office is considering the claims in a manner that is inconsistent with the specification.

Summarily, applicants respectfully submit that rat gamma B crystallin, human gamma B crystallin, and mouse gamma B crystallin would not be understood by one of ordinary skill in the art to be "mutagenized" as taught in the instant specification and recited in the instant claims. Furthermore, rat gamma B crystallin, human gamma B crystallin, and mouse gamma B crystallin do not possess a new or improved antigen binding specificity as recited in claim 1. Accordingly, applicants respectfully submit that the GENBANK® Accession Nos. cited by the Patent Office do not support a rejection of claim 1 under 35 U.S.C. § 102(b).

Therefore, applicants respectfully submit that claim 1 has been distinguished over the cited GENBANK® Accession Nos. Furthermore, claims 2, 7-12, 26-28, and 42 are also believed to be distinguished from the cited GENBANK® Accession Nos. based on their direct or indirect dependence from claim 1. Claim 2 has been canceled, and thus the instant rejections are moot as to claim 2. Applicants respectfully submit that claims 1, 7-12, 26-28, and 42 are in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

VII. Discussion of the New Claim

Claim 46 has been added. Claim 46 recites a protein with beta-sheet structure, wherein amino acids on a surface of the protein located in at least two β-strands of at least one beta sheet are mutagenized, wherein the mutagenizing is selected from the group consisting of an insertion, a deletion, a substitution, and combinations thereof, such that the protein has a new or an improved antigen binding specificity and wherein the protein to be mutagenized is selected from the group consisting of a crystallin, a spheruline, a heat shock protein, a cold shock protein, a β-helix protein, and a fibronectin, with the proviso that:

(i) the protein without substitution, deletion, or insertion has no binding activity at the surface of the beta-sheet structure wherein the amino acids are mutagenized, and after substitution, deletion, or insertion at the surface of the beta-sheet structure, the protein has a new antigen binding specificity, or

(ii) the protein has a binding activity before the substitution, deletion, or insertion, and that after the substitution, deletion, or insertion at the surface of the beta-sheet structure, the protein has an additional new or an improved binding activity;

and further wherein said protein is prepared by a method comprising:

(a) selecting a protein from the group consisting of a crystallin, spherulin, a heat shock protein, a cold shock protein, a β-helix protein, and fibronectin;

(b) selecting a binding partner of the protein;

(c) mutagenizing a nucleic acid molecule encoding amino acids on a surface of the protein located in at least two β-strands of at least one beta-sheet of the protein with beta-sheet structure, wherein the mutagenizing is selected from the group consisting of an insertion, a deletion, a substitution, and combinations thereof;

(d) expressing the mutagenized nucleic acid molecule of step (c) in order to produce the mutagenized protein;

(e) contacting the mutagenized protein with said binding partner of step (b); and

(f) selecting and isolating of a mutagenized protein with a new or improved binding activity towards the binding partner of step (b).

Support for the new claim can be found throughout the specification as filed, including particularly in the claims as filed (*e.g.*, claims 1 and 21 as originally filed). Additional support can be found on page 6, lines 11-18 (exemplary proteins); page 8, lines 2-8 (selecting binding partners); page 8, line 15, through page 9, line 5 (mutagenesis); page 10, lines 4-5 (expression of the mutagenized protein); and original claim 24 (contacting the mutagenized protein and the binding partner and isolating mutagenized proteins). Thus, applicants respectfully submit that new claim 46 includes no new matter.

Applicants respectfully submit that the remarks presented hereinabove with respect to the rejections under 35 U.S.C. § 102 are equally applicable to new claim 46. Specifically, applicants respectfully submit that none of the cited references teaches a mutagenized protein selected from the group consisting of a crystallin, spherulin, a heat shock protein, a cold shock protein, a β-helix protein, and fibronectin, wherein the

mutagenized protein gains a new or improved or an additional new or improved binding activity as a result of the mutagenesis.

As a result, applicants respectfully submit that claim 46 is in condition for allowance, and respectfully solicit a Notice of Allowance to that effect.

CONCLUSIONS

Should there be any minor issues outstanding in this matter, the Examiner is respectfully requested to telephone the undersigned attorney. Early passage of the subject application to issue is earnestly solicited.

Deposit Account

The Commissioner is hereby authorized to charge any fees associated with the filing of this correspondence to Deposit Account Number **50-0426**.

Respectfully submitted,

JENKINS, WILSON & TAYLOR, P.A.

Date: 01/10/2006 By: _____

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Exhibit B